Reference: *Biol. Bull.*, 143: 617–622. (December, 1972)

# OBSERVATIONS ON THREE SPECIES OF JELLYFISHES FROM CHESAPEAKE BAY WITH SPECIAL REFERENCE TO THEIR TOXINS. II. *CYANEA CAPILLATA*[1]

NOLAN E. RICE AND W. ALLAN POWELL

*Department of Biology and Department of Chemistry, University of Richmond, Virginia 23173*

*Cyanea capillata*, the pink jellyfish or lion's mane, occurs in Chesapeake Bay and its tributary rivers from the latter part of November to the first of May. The largest specimens attain an umbrellar diameter of eight inches, but most specimens observed are in the range of four to six inches. Pigmentation varies from pink to brown in different specimens. Compared to *Chrysaora quinquecirrha* the much more numerous tentacles of *Cyanea* are vastly shorter even when fully extended. The anatomical features of the Chesapeake Bay *Cyanea* are identical in every respect to those of the North Atlantic form for which a record eight feet umbrellar width (Miner, 1950) has been reported.

Cleland and Southcott (1965) detail reports by various observers (Wood, 1874; Kristenson, 1949; Uvnäs, 1960) on the stinging ability of *C. capillata*, and other reports (Pope, 1953a, 1953b; Barnes, 1960) of stingings by *C. annaskala*. On the other hand Burnett (1971) reports that the smaller *C. capillata* of Chesapeake Bay (page 70) "rarely produce symptomatic stings in humans."

Although Mayer (1910) believes that *C. capillata* and *C. annaskala* are distinct northern and southern hemispheric species with many intergrading varieties in each, Kramp (1961), however, states that there is only one species of *Cyanea*, namely *C. capillata* which is cosmopolitan in distribution.

Halstead (1965), in a table adapted from several sources, lists three distinct types of nematocysts from *Cyanea*. Cleland and Southcott (1965), however, find two, and perhaps three, different types in *Cyanea* sp. (*C. annaskala* ?). Burnett (1971) describes two types of nematocytes (cnidoblasts) in the tentacles of the Chesapeake Bay *Cyanea*. Presumably these give rise to the mature types of nematocysts found in adults.

Rice and Powell (1970) reported observations on *Chrysaora quinquecirrha* related to (1) the extraction and isolation of nematocyst toxin; (2) toxicity experiments; and (3) the determination of the chemical nature of the toxins. The present paper presents our observations on *C. capillata* along these same lines. Reference may be made to the earlier paper for literature which might be relevant here as well.

## Materials and Methods

Jellyfishes were collected in the Piankatank River near Deltaville, Virginia during the months of January, February, March and April, 1967–1971. Because

[1] This investigation was supported by several Faculty Research Grants of the University of Richmond and a Virginia Academy of Science Research Grant.

it was difficult and tedious to secure tentacles only, entire animals were used. Although this entailed the processing of much unwanted material, nematocysts from the oral lobes and exumbrella were obtained in addition to those on the tentacles.

Nematocyst toxin was extracted by the same method that was used for *Chrysaora* (Rice and Powell, 1970). This involved the following processes: autolysis of the material in the refrigerator; screening through nylon netting and silk bolting cloth; sedimentation; decanting the supernatant; centrifugation and washing of the nematocysts; homogenization of the nematocysts; centrifugation at 18,500 rpm; and subsequent collection of the supernatant containing the toxin.

Since a rather large amount of lipid material was encountered as a result of using whole animals, extensive centrifugation and washing was necessary to remove the lipid associated with the nematocysts.

White Swiss mice (Wistar strain) were used as test animals in all of the toxicity experiments. All materials were suspended in 0.9% NaCl solution and injected intraperitoneally in 1 ml doses. Mice injected with 0.9% NaCl solution served as controls.

The following standard reagents were used for chemical tests: Biuret, ninhydrin, Molisch, and Benedict's. Van Gieson's picrofuchsin and Mallory's aniline blue strains were applied to nematocysts.

The electrophoresis pattern of the nematocyst toxin was obtained using polyacrylamide gels. The standard separating gel of 7% acrylamide provided by Canalco Instrument Corporation was used at a pH of 9.5 according to their instructions for disc electrophoresis.

## Results

The stinging ability of the Chesapeake Bay *Cyanea* was tested by using mature volunteers of both sexes and various ages up to sixty years. When a living tentacle was pressed tightly with a glass slide against the biceps area of the arm no reaction was observed in 42 subjects. Six, however, thought they felt a faint stinging sensation. When faradic shock was applied to a tentacle, the 6 subjects tested were unaffected. Since jellyfish stingings usually take place in the water, and water softens the skin, experiments were performed after the biceps area of both arms was subjected to a 1.5% salt water solution for 15 minutes. One arm served as a control. Twenty subjects were unaffected; 15 subjects reported a faint tingling sensation. In only a few cases was mild erythema observed.

The tentacles of *Cyanea* are covered with almost contiguous batteries of nematocysts, each battery consisting of from 2 to 4 dozen or more nematocysts. The batteries near the base of each tentacle are only about half the size of the distal ones. Batteries also occur on the marginal portions of the oral lobes with scattered nematocysts elsewhere. There are no nematocysts on the subumbrellar surface except for some relatively small batteries on the folds surrounding the gonads. Scattered batteries occur on the exumbrellar marginal areas of the lappets. Toward the center of the bell these give way to occasional isolated individual nematocysts.

Three types of nematocysts were observed: large atrichous isorhizas; medium sized heterotrichous microbasic euryteles; and small holotrichous isorhizas. More

than half of the nematocysts in a tentacle battery are euryteles and, with the exception occasionally of one or two atrichous isorhizas, the remainder are holotrichous isorhizas. The capsules of the atrichous isorhizas ranged in length from 15 to 20 $\mu$ and in width from 8 to 12 $\mu$. The discharged tubes of these measured from 500 to 1400 $\mu$ in length. The capsules of the heterotrichous microbasic euryteles ranged in length from 8 to 13 $\mu$ and in width from 5 to 8 $\mu$. The tubes measured from 100 to 400 $\mu$ in length. The butts were 8 to 13.0 $\mu$ in length. The capsules of the holotrichous isorhizas ranged in length from 6 to 8 $\mu$ and in width from 4 to 5 $\mu$. The tubes measured from 50 to 200 $\mu$ in length. The tubes of all three types are of the order of magnitude of 0.5 $\mu$ in width.

The discharge of nematocysts from living tentacles was observed microscopically after the application of pressure, faradic electrical shock and chemical reagents such as formalin-acetic-alcohol (FAA). However, these were ineffective on isolated nematocysts. When an entire living jellyfish in sea water was stimulated by electrical shock, it contracted convulsively. Nematocysts on a few tentacles in line with the flow of current discharged but those on most of the tentacles did not. If isolated tentacles were stimulated, large numbers of nematocysts discharged but many were unaffected. Continued shocks in the same region had no further effect.

Homogenization of heavy suspensions of nematocysts in the frozen state was effective in rupturing 60–80% of them.

The penetration power of nematocysts was studied using Grenacher's borax-carmine agar (2%) as described in an earlier paper (Rice and Powell, 1970). When living tentacles in contact with the agar were stimulated by the application of FAA, nematocysts were discharged to various distances into the gel. Numerous measurements showed that the vast majority of tubes penetrated the agar a distance of from 200 to 350 $\mu$, a few up to 400 $\mu$, and an occasional one up to 1 mm. The paths of tubes through the agar were sinuous, few were straight. Many of the tubes were coiled on themselves.

The following materials were used in toxicity experiments: the supernatant after centrifugation of homogenized nematocyst suspensions at 18,500 rpm for one hour; washed undischarged capsules; washed capsular debris and tubes; and washed lipid collected from the surface of supernatant. Injections of supernatant (minus lipid) into mice were quite toxic. The LD 50, based on air dried residues corrected for salt content, was of the order of magnitude of 6 $\mu$g/g of body weight. Washed capsular debris and tubes had no observable effect on mice, even in heavy, milky suspensions. When washed undischarged nematocysts in moderately heavy suspensions were injected, the mice seemed not to be affected; heavier suspensions, however, were fatal. Lipids in heavy suspension had no apparent toxic effect.

All of these materials except the lipid were subjected to the following reagents: Biuret, ninhydrin, Benedict's, and Molisch. The washed undischarged nematocysts gave a positive Biuret test and the supernatant was Biuret and ninhydrin positive. All other tests were negative. The positive tests show that the toxin is contained in the nematocysts and that it is proteinaceous.

Electrophoresis studies of the supernatant using polyacrylamide gel and subsequently staining with Coomassie blue, confirm the protein nature of the toxin.

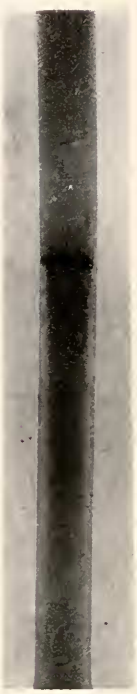

FIGURE 1. Electrophoretogram of the toxin of *Cyanea* nematocysts obtained using polyacrylamide gels with subsequent staining with Coomassie blue stain for protein; origin at the top of the figure.

Figure 1 is a photograph of a typical electrophoretogram. A single, sharply defined band indicates that the toxin is a single protein.

Whatever the chemical nature of nematocyst capsules and tubes, staining with aniline blue or picrofuchsin, accepted vertebrate collagen stains, was negative.

## DISCUSSION

As noted by Burnett (1971) and as observed by us independently in experiments reported here, the Chesapeake Bay *Cyanea* is incapable of stinging most individuals. Any reaction that does occur is so mild and so short-lived as to be barely noticeable. These observations are in strong contrast to reports by others (Cleland and Southcott, 1965) who report painful experiences with Mayer's (1910) northern and southern hemispheric *Cyanea* which Kramp (1961) maintains belong to the same species.

Although the reason for the difference in stinging ability of the Chesapeake Bay *Cyanea* and that found elsewhere is not known, it hardly seems likely that it is due to its smaller size. Unpublished observations on *Chrysaora quinquecirrha* show, that for equal surface areas of skin affected, small specimens produce just as severe a reaction as do larger ones. It may be that the difference in stinging ability is due to a difference in the power of penetration of the nematocysts. Although the Chesapeake Bay *Cyanea* and those found elsewhere have the same types

of nematocysts, it may be that the Chesapeake form has a much lower percentage of the type which has the power to penetrate the skin. Some evidence of a difference in penetrating powers of nematocysts of *Chrysaora* and *Cyanea* has been observed in our laboratory.

A comparison of these two species shows that: (1) nematocyst batteries of *Chrysaora* consist mostly of atrichous and holotrichous isorhizas and fewer euryteles whereas batteries of *Cyanea* are made up of euryteles and holotrichous isorhizas and few atrichous isorhizas; (2) the atrichous isorhiza tubes of *Chrysaora* have a maximum length of 2 mm compared to 1.4 mm in *Cyanea;* and (3) the paths of penetration of tubes of *Chrysaora* into agar are straight while those of *Cyanea* are sinuous and often coiled on themselves. These facts would seem to indicate that most, if not all, of the effective toxin in *Chrysaora* stings comes from the atrichous isorhizas, which may penetrate deeper into the skin and which are present in larger numbers as compared to the same type in *Cyanea*, and consequently explain why the latter rarely produces any symptoms.

Electric shock has been used successfully to effect discharge of nematocysts from living Cnidaria (Kline and Waravdekar, 1960; Barnes, 1967). We found faradic shocks just as effective in producing discharge of nematocysts in *Cyanea* as in *Chrysaora* (Rice and Powell, 1970). The same was also true of FAA fixative.

As in *Chrysaora*, both of the above agents were ineffective in causing discharge of isolated nematocysts although Phillips and Abbott (1957) were successful in obtaining by various chemical means the discharge of nematocysts of *Metridium senile fimbriatum*. Rupture or discharge of the nematocysts of *Cyanea* was not produced to any observable degree by shock or FAA treatment which is in agreement with similar results for *Chrysaora*. This contrasts with the work of Burnett, Stone, Pierce, Cargo, Layne and Sutton (1968) who had positive results with a number of physical and chemical agents. Homogenization of frozen nematocysts of *Cyanea* caused the rupture of 60 to 80%.

It has been stated earlier that the paths of the nematocyst tubes of *Cyanea* in agar were sinuous as compared to the straight paths of *Chrysaora*. No confirmed explanation for this difference has been formulated. It could be due to differences in rigidity, size, shape, or force of discharge of the tubes.

In *Cyanea*, as in *Chrysaora*, the evidence shows that: (1) the toxic principle is contained in the capsules of the nematocysts; (2) it is protein; (3) the capsules and tubes are not toxic; (4) associated lipid is not toxic; and (5) the chemical nature of the capsules and tubes is unlike that of vertebrate collagen.

The LD 50 (6 μg/g) of the *Cyanea* toxin as compared to that of *Chrysaora* (16–19 μg/g) indicates a greater toxicity of the former for mice.

We are indebted to the following persons for assistance: Mr. William A. Dorsey, Chief of Public Laboratories, Richmond City Health Department for supplying us with white mice; Dr. Wilton R. Tenney, Department of Biology, University of Richmond for the photograph of the electrophoretogram; and Dr. Francis B. Leftwich, Department of Biology, University of Richmond for technical assistance.

## Summary

1. The human body exhibits little or no response to contact with the tentacles of specimens of *Cyanea capillata* from Chesapeake Bay.

2. Three types of nematocysts were identified: atrichous isorhizas, holotrichous isorhizas, and heterotrichous microbasic euryteles.

3. The tubes of nematocysts discharged into 2% agar take a sinuous course, often coiling on themselves. Most tubes penetrated less than 350 $\mu$.

4. Although faradic shock and FAA caused discharge of nematocysts from living tentacles, they failed to produce discharge of isolated nematocysts.

5. Homogenization of thoroughly washed frozen nematocysts causes rupture, releasing the toxic principle which is a single protein.

6. The LD 50 of the toxin was of the order of magnitude of 6 $\mu$g/g of mouse body weight.

7. Mice were unaffected by intraperitoneal injections of washed capsular debris and tubes, or lipids, or of washed undischarged nematocysts in moderately heavy suspensions.

## LITERATURE CITED

Barnes, J. H., 1960. Observations on jellyfish stingings in north Queensland. *Med. J. Aust.*, **2**: 993–999.

Barnes, J. H., 1967. Extraction of cnidarian venom from living tentacle. Pages 115–129 in F. E. Russell, and P. R. Saunders, Eds., *Animal Toxins*. Pergamon Press, Oxford.

Burnett, J. W., J. H. Stone, L. H. Pierce, D. G. Cargo, E. C. Layne and J. S. Sutton, 1968. A physical and chemical study of sea nettle nematocysts and their toxin. *J. Invest. Dermatol.*, **51**: 330–336.

Burnett, J. W., 1971. An electron microscopic study of two nematocytes in the tentacle of *Cyanea capillata*. Chesapeake Sci., **12**: 67-71.

Cleland, J. B., and R. V. Southcott, 1965. Injuries to man from marine invertebrates in the Australian region. *Australia National Health and Medical Research Council Special Report Series, No.* **12**: 1–282.

Halstead, B. W., 1965. *Poisonous and Venemous Marine Animals of the World*. U. S. Government Printing Office, Washington, 994 pp.

Kline, E. S., and V. S. Waravdekar, 1960. Inhibitor of succinoxidase activity from *Hydra littoralis*. *J. Biol. Chem.*, **235**: 1803–1808.

Kramp, P. L., 1961. Synopsis of the medusae of the world. *J. Mar. Biol. Ass. U. K.*, **40**: 1–469.

Kristenson, A., 1949. Some observations concerning the pathophysiological effects on the human skin caused by the stinging jellyfish (*Cyanea capillata*). *Acta Physiol. Scand.*, **18**: 151–156.

Mayer, A. G., 1910. *Medusae of the World*, Volume 1. Carnegie Institution of Washington, Washington, D. C., 735 pp.

Miner, R. W., 1950. *Field Book of Seashore Life*. Putnam, New York, 888 pp.

Phillips, J. H., and D. P. Abbott, 1957. Isolation and assay of the nematocyst toxin of *Metridium senile fimbriatum*. *Biol. Bull.*, **113**: 296–301.

Pope, E. C., 1953a. Sea lice or jellyfish? *Aust. Mus. Mag.*, **11**: 16-21.

Pope, E. C., 1953b. Marine stingers. *Aust. Mus. Mag.*, **11**: 111–115.

Rice, N. E., and W. A. Powell, 1970. Observations on three species of jellyfishes from Chesapeake Bay with special reference to their toxins. I. *Chrysaora (Dactylometra) quinquecirrha*. *Biol. Bull.*, **139**: 180–187.

Uvnäs, B., 1960. Mechanism of action of a histamine-liberating principle in jellyfish (*Cyanea capillata*). *Ann. New York Acad. Sci.*, **90**: 751–759.

Wood, J. J., 1874. *Out of Doors. A Selection of Original Articles on Practical Natural History*. Longman, Green and Co., London, 342 pp.